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VERIFICATION OF A TRANSLATION

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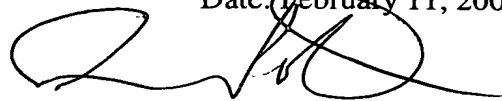
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Date: February 11, 2002

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Process for encapsulating active materials by  
coacervation of polymers in non-chlorinated organic  
solvent

- 5 The present invention relates to a process for  
microencapsulating an active principle by coacervation,  
used especially for preparing sustained-release  
pharmaceutical forms.
- 10 Microencapsulation techniques are conventionally used  
for separating incompatible chemical substances, for  
converting liquids into powders, for improving the  
bioavailability of active principles, for masking the  
unpleasant taste or odor of certain compounds and for  
15 preparing sustained-release pharmaceutical forms.

Sustained-release pharmaceutical forms may be  
administered subcutaneously or intramuscularly and may  
be found directly in the blood flow or close to the  
20 organ to be treated, and as such biodegradable polymers  
are often chosen to form part of their composition.

Sustained-release systems based on biodegradable  
polymers may be administered parentally without removal  
25 by surgical operation, since the biodegradable polymers  
become converted in the body into metabolites that are  
eliminated via the natural routes. The active principle  
is released according to kinetics that are modulated by

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the diffusion of the active principle and the degradation process of the polymer. Furthermore, the patient's compliance with the treatment is improved since a less frequent administration is involved.

5

Among the biodegradable polymers frequently used in the encapsulation of active principles are poly( $\alpha$ -hydroxy acids), especially polylactic acids (PLAs) and polylactic acid-glycolic acid (PLAGAs), poly-  
10  $\epsilon$ -caprolactone, polyorthoesters, for instance Chronomer<sup>®</sup> and Alzamer<sup>®</sup>, polyanhydrides, especially the copolymer of sebacic acid and of (carboxyphenoxy)propane, and biodegradable natural  
15 polypeptides and polymers, for instance albumin, bovine serum albumin, collagen and chitosan.

Two main types of microencapsulation technique exist:

- solvent-free techniques, for instance spray-  
20 congealing, extrusion (coextrusion/spheronization), gelation, prilling and precipitation of supercritical solutions (RESS), and
- solvent techniques, for instance nebulization,  
25 coacervation, emulsion-evaporation, emulsion-extraction, and variants thereof starting with water/oil/water double emulsions.

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- The prolonged contact of sustained-release pharmaceutical forms with an aqueous medium justifies the use of polymers of more or less hydrophobic nature, which are thus mainly soluble in an organic medium.
- 5 However, biodegradable polymers based on controlled-release systems are poorly soluble in solvents of low potential toxicity (category 3 solvents according to the ICH standard).
- 10 Accordingly, the standard microencapsulation techniques (coacervation and emulsion-evaporation) essentially use chlorinated solvents, for instance dichloromethane (category 2 solvent according to the ICH standard, i.e. solvent to be limited), as solvent for the polymer.
- 15 However, it is a chlorinated solvent that is known for its neurotoxicity. The permitted residual level of dichloromethane in the finished product is 600 ppm according to standard ICH4.
- 20 Whichever microencapsulation technique is used, the microparticles obtained contain residual amounts of solvents. It thus appears to be necessary to develop novel microencapsulation methods that do not involve chlorinated solvents. Two major solutions arise for
- 25 solving this problem.


One solution for achieving encapsulation without chlorinated solvents is based on methods using no solvent, but certain polymers cannot be used according

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to these methods. In addition, the properties of the particles obtained by these methods do not necessarily meet the demands of a long-term treatment.

5 Another solution consists in replacing chlorinated solvents with non-toxic solvents. The microencapsulation methods which use chlorinated solvents have been widely studied and the variables are known. However, the replacement of chlorinated solvents with  
10 nonchlorinated solvents modify the physicochemical interactions between the various components of the formulation. The behavior of biodegradable polymers in the replacement solvents is very different from that in the chlorinated solvents. Thus, poly(L-lactide) and  
15 poly(D,L-lactide) are insoluble in ethyl acetate or acetone and no polymer is soluble in ethanol, which is, however, a favored solvent since it is of low toxicity.

The present invention proposes a process of  
20 microencapsulation by coacervation that does not use any chlorinated solvent. More specifically, the invention relates to a process of coacervation by addition of nonsolvent. Coacervation by addition of nonsolvent requires the use of three miscible solvents;  
25 one of these three solvents is a solvent for the polymer and the other two are nonsolvents for the polymer.



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The principle of the coacervation is based on the controlled desolvation of a polymer dissolved in an organic solvent containing an active principle generally in particulate form, induced by addition of a  
5 nonsolvent or a polymer-coacervating agent. The solubility of the polymer in the organic solvent is lowered and two immiscible phases form: the coacervate gradually settles out at the surface of the active principle. The addition of a curing agent allows the  
10 formation of a continuous polymer film around the active principle.

The active principle particles may be liquid or solid. The active principle may also be initially dissolved in  
15 the solvent for the polymer. In this case, it reprecipitates in particulate form when the coacervating agent is added, or can form a homogeneous solid solution in the polymer particles derived from the coacervation.

20

Study of the polymer/solvent/coacervating agent interactions, for each combination, makes it possible to produce a phase diagram so as to define the ideal polymer/solvent/coacervating agent ratio required for  
25 efficient encapsulation. However, it is difficult to predict the encapsulation of an active principle, since the interface properties, in relation with the molecular interactions between the polymer, the solvent and the coacervating agent, change constantly with the

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composition of the coacervate (Thomassin C.,  
Merkle H.P., Gander B.A., Physico-chemical parameters  
governing protein microencapsulation into biodegradable  
polyester by coacervation, Int. J. Pharm., 1997, 147,  
5 173-186).

The major problem of the coacervation technique is the  
possible aggregation of particles. In an attempt to  
solve this, authors have proposed reducing the  
10 temperature of the system, essentially in the curing  
step. The walls are then solid enough to prevent  
adhesion. Solutions such as the use of chlorofluoro-  
carbons (CFCs) or reduction of the temperature cannot  
be transposed to the industrial scale. On the other  
15 hand, the use of silicone oil is capable of stabilizing  
the system by virtue of its viscosity (Ruiz J.M.,  
Tissier B., Benoit J.P., Microencapsulation of peptide:  
a study of the phase separation of poly(D,L-lactic  
acid-co-glycolic acid) copolymers 50/50 by silicone oil,  
20 Int. J. Pharm., 1989, 49, 69-77).

Despite high residual contents of solvents, the  
coacervation technique remains a technique of choice  
for the encapsulation of fragile active principles, and  
25 especially of water-soluble active principles, in  
nonaqueous medium.

The choice of the solvent/coacervating agent/curing  
agent combinations is guided by various criteria:

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- the solvent must dissolve the polymer; it is preferable for it not to dissolve the active principle, although the process can still be used with an active principle that is soluble in the solvent for the polymer;
- the coacervating agent must be miscible with the solvent for the polymer. It must not be a solvent for the polymer, otherwise this would amount to a simple transfer of the polymer from the solvent to the coacervating agent. Furthermore, it must not dissolve the active principle, in order to limit the encapsulation losses;
- the curing agent must be partially miscible with the solvent for the polymer, so as to facilitate the extraction. It must not dissolve either the polymer or the active principle, otherwise the encapsulation yield would be greatly reduced.

In the prior art, the coacervation technique uses dichloromethane or chloroform as solvent for the polymer, a silicone oil as coacervating agent and heptane as curing agent.

The present invention relates to a process for microencapsulating an active principle by coacervation, which consists of



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- the controlled desolvation or coacervation of a polymer dissolved in an organic solvent containing said active principle, said coacervation being  
5 induced by addition of a nonsolvent and being reflected by the deposition of the polymer at the surface of said active principle, and then

- the curing of the polymer deposit by addition of a  
10 curing agent, said curing being reflected by the formation of a continuous film coating said active principle,

characterized in that

15

- the solvent for the polymer is a nonchlorinated organic solvent with a boiling point of between 30°C and 240°C and a relative dielectric permittivity of between 4 and 60, advantageously  
20 chosen from ethyl acetate, N-methylpyrrolidone, methyl ethyl ketone, acetic acid and propylene carbonate, and mixtures thereof,

- the nonsolvent is an alcohol or a ketone  
25 containing 2 to 5 carbon atoms and preferably 2 or 3 carbon atoms, in particular ethanol ( $\epsilon=24$ ), 2-propanol ( $\epsilon=18$ ), 1,2-propanediol ( $\epsilon$  between 18 and 24) and glycerol ( $\epsilon=40$ ), or methyl ethyl ketone ( $\epsilon=18$ ),

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- the curing agent is chosen from water, alcohols containing 1 to 4 carbon atoms, on condition that the curing agent is an alcohol that is different than the nonsolvent, and mixtures thereof.

Although N-methylpyrrolidone is of category 2, like dichloromethane, its limit concentration is markedly higher (4840 ppm as opposed to 600 ppm for dichloromethane).

Advantageously, the nonsolvent and the curing agent are chosen, respectively, from the following pairs: 1,2-propanediol and 2-propanol, glycerol and 1,2-propanediol, glycerol and 2-propanol, 2-propanol and 1,2-propanediol.

According to one preferred embodiment, the polymer is a biodegradable polymer with a weight-average molecular mass (Mw) of between 10,000 and 90,000 g/mol and preferably between 15,000 and 50,000 g/mol, and with a polydispersity index (Ip) of between 1 and 3.5 and preferably between 1.5 and 2.5.

A certain number of other additional characteristics will be indicated below to illustrate various preferred embodiments of the invention.

- 10 -

According to these additional characteristics, the polymer is a lactic acid polymer (PLA) or a copolymer of lactic acid and of glycolic acid (PLAGA).

- 5 The polymer is a PLAGA such that  $M_w$  is between 15,000 and 20,000 g/mol, preferably equal to 17,500,  $I_p$  is between 1 and 2, preferably equal to 1.6, and the percentage of glycolic acid is less than 30%, preferably equal to 25%.

10

The polymer concentration in the solvent is between 1% and 10% (w/v) and preferably about 4% (w/v).

15

The nonsolvent/solvent ratio by volume is between 1/2 and 1/1.

20

The coacervation temperature is less than the glass transition temperature of the polymer, preferably less than or equal to 25°C, preferably less than 4°C and more preferably less than or equal to -4°C.

The nonsolvent is added in successive doses from 200  $\mu$ l to 1 ml.

- 25 The coacervation is performed with stirring, for example with magnetic stirring, at a speed of between 200 and 1000 rpm.

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The curing agent also contains a surfactant, the concentration of said surfactant in the curing agent being between 0.5% and 10% (v/v).

- 5 The surfactant is a sorbitan ester, for example Tween<sup>®</sup> 80, or polyvinyl alcohol.

The curing agent/solvent ratio by volume is between 5/1 and 180/1 and preferably between 15/1 and 120/1.

10

The microspheres are cured with stirring, for example magnetic stirring at a speed of between 500 and 1500 rpm.

- 15 The curing temperature is less than or equal to 25°C, preferably less than 4°C and more preferably less than or equal to 0.5°C.

The curing agent is added in several portions and  
20 preferably in at least four portions.

The curing lasts between 2 and 4 hours.

The microparticles obtained after the curing operation  
25 are filtered through Millipore<sup>®</sup> system, by centrifugation or through fluted paper.

When the active principle forms a dispersion in the solution for the polymer, the solvent and the

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nonsolvent have a viscosity that is high enough to stabilize the active principle.

The particle size of the active principle is between 1  
5 and 50 microns and preferably between 5  $\mu\text{m}$  and 30  $\mu\text{m}$ .

According to one preferred embodiment, the solvent is N-methylpyrrolidone, the nonsolvent is ethanol and the curing agent is water.

10

According to another preferred embodiment, the solvent is ethyl acetate, the nonsolvent is 2-propanol and the curing agent is water. The polymer is a 75:25 PLAGA such that the Mw is between 15,000 and 20,000 and  
15 preferably equal to 17,500, and the Ip is between 1 and 2 and preferably equal to 1.6.

According to a third preferred embodiment, the solvent is acetic acid, the curing agent is water and the  
20 polymer is a 50:50 PLAGA.

In the context of the present invention, when the active principle is insoluble in the solvent for the polymer, either a suspension or an emulsion is  
25 prepared.

To prepare the suspension, the active principle is ground using a mortar and then placed in suspension in the solvent. The suspension may be homogenized by

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magnetic stirring: the coacervation is then also performed with magnetic stirring. The suspension may also be homogenized with mechanical stirring, at variable speed (impeller stirrer, Heidolph RGL500, 5 Prolabo, Paris, France) or using an Ultra-Turrax® T25 mixer (Prolabo, Paris, France). In these two latter cases, the coacervation is then performed with mechanical stirring.

- 10 Dispersing the active principle in the polymer solution may also be conventionally performed with ultrasound agitation.

When the active principle is water-soluble, to prepare 15 the emulsion, the active principle is dissolved in water and the water/solvent emulsion of the polymer is then prepared with mechanical stirring. The coacervation then takes place with mechanical stirring.

- 20 When the active principle is soluble in the solvent for the polymer, the coacervation is performed with mechanical stirring.

In the context of the present invention, the polymer is 25 a biodegradable polymer frequently used in the encapsulation of active principles, preferably a PLA or a PLAGA, more preferably a PLAGA with a weight-average molar mass ( $M_w$ ) of between 10,000 and 90,000, a number-average molar mass ( $M_n$ ) of between 4000 and 40,000, a

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polydispersity index ( $I_p$ ) of between 1 and 3.5, and for which the proportion of glycolide is between 10 and 60%. Polymers with a high weight-average molar mass of greater than or equal to 15,000 g/mol will be preferred, since they allow the manufacturing yield to be increased by increasing the volume of the coacervate phase. Polymers with a low polydispersity index ( $I_p \leq 2.6$ ) will also be preferred, since the fractions of low molecular weight remain in solution and bring about a reduction in yield, or cause the aggregation of the microparticles by sticking to their surface.

The PLAGA is, for example, Resomer<sup>®</sup> RG 502 (Boehringer Ingelheim,  $M_w = 14,300$  g/mol),  $M_n$  6900 g/mol,  $I_p$  2.5, 50% glycolide), Resomer<sup>®</sup> RG 756 ( $M_w$  89,800 g/mol,  $M_n = 35,200$  g/mol,  $I_p$  2.6, 25% glycolide), Resomer<sup>®</sup> RG 858 ( $M_w = 87,000$  g/mol,  $M_n$  22,000 g/mol,  $I_p$  3.9, 15% glycolide), Phusiline supplied by Phusis ( $M_w = 17,500$  g/mol),  $M_n$  10,940 g/mol,  $I_p$  1.6, 25% glycolide).

The polymer concentration in the solvent must be sufficient to increase the viscosity of the medium, which makes it possible to stabilize the dispersed coacervate droplets and to limit their aggregation, on the one hand, and to reduce the formation of small-sized microparticles, on the other hand.

- 15 -

The polymer concentration is preferably between 1 and 10% (w/v) and more preferably equal to about 4% (w/v).

The viscosities of the solvent and the nonsolvent must  
5 be sufficient to stabilize the coacervate droplets.

The volume of nonsolvent to be added is defined so as to bring the system into the window of stability and to obtain a stable coacervate. However, the volume of  
10 nonsolvent also depends on the concentration of the active principle crystals in suspension in the organic solution of polymer.

The addition of an excess of nonsolvent makes it  
15 possible to accelerate the curing of the wall of the microparticles, to prevent their aggregation and to improve the extraction of the solvent.

The rate of addition of the nonsolvent is low enough to  
20 prevent the formation of a large number of excessively small microparticles, i.e. between 1 and 2  $\mu\text{m}$  in size. In addition, the slower the phase separation, the more uniform the particle size distribution of the microparticles and the smoother the surface of the  
25 microparticles. The addition of nonsolvent preferably takes place gradually in doses from 200  $\mu\text{l}$  to 1 ml, waiting for at least one minute between each dose.



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Reducing the stirring speed during the coacervation step increases the size of the coacervate droplets and then of the final microparticles. However, below a limit speed, which varies depending on the systems, the kinetics of deposition of the coacervate are too slow and/or the coacervate droplets are too large and not sufficiently stable. A mechanical or magnetic stirring of between 200 rpm and 1000 rpm often gives good results.

10

The temperature is the essential parameter of the coacervation; it must be less than the glass transition temperature of the polymer. The lower the temperature, the more viscous the medium and the less the microparticles are prone to aggregate.

15

The ideal curing agent should not dissolve either the active principle or the polymer. It should readily extract the solvent for the polymer. The curing agent used is water optionally supplemented with surfactant or an alcohol. Water advantageously allows the solvent for the polymer to be readily extracted. It also has the advantage of being cheap and of not requiring reprocessing of the effluents. However, water is not the ideal curing agent in the case of water-soluble active principles, since any prolonged contact is responsible for diffusion of the active principle, which is reflected by a low degree of encapsulation.

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When the encapsulated active principle is hydrophilic during the curing, it is rapidly dissolved by the water, which penetrates into the microparticles, and can back-diffuse out of the particles. By working at  
5 low temperature, the diffusion phenomena are reduced, and thus the losses of active principle toward the aqueous phase are reduced, and the encapsulation yield is improved.

10 Other possibilities may be envisaged for reducing the diffusion of the active principle; for instance saturation of the outer phase with an electrolyte or the active principle itself, if it is cheap, and combining water with another solvent that has strong  
15 affinity for the solvent for the polymer, so as to extract microspheres. Thus, the volume of water used is reduced and the contact with water is attenuated.

The surfactant or the alcohol make it possible to limit  
20 the self-aggregation of the microparticles so as to form a homogeneous dispersion. They have been selected on the basis of their harmlessness. The surfactants are chosen from those commonly used in formulations for injection such as polyoxyethylenated sorbitan esters,  
25 for instance Tween® 80 and Tween® 20 (hydrophilic surfactants).

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Montanox<sup>®</sup> 80 (polyoxyethylenated sorbitan monooleate) is a hydrophilic emulsifier that may be used in the composition of an emulsion of oil/water type.

5 Montane<sup>®</sup> 80 (sorbitan oleate) is its equivalent in the lipophilic surfactant range. Solutol<sup>®</sup> HS 15 (polyethylene glycol 660 hydroxystearate) is a nonionic surfactant of hydrophilic nature used in injectable solutions.

10

Synperonic<sup>®</sup> PE/F 68 (Poloxamer 188) is a block copolymer of polyoxyethylene and of polyoxypropylene.

15 Finally, polyvinyl alcohol has been used in two different grades: Mowiol<sup>®</sup> 4/88 and Rhodoviol<sup>®</sup> 4/125.

The volume of curing agent is based on a compromise. It must be sufficient to rapidly remove the solvent for the microspheres, but it will have to limit the  
20 diffusion of the active principle out of the microspheres. The volume is defined from the solubility criteria of the solvent in the outer phase, such that the final concentration of the solvent in the curing agent is less than the saturation concentration of  
25 solvent in the outer phase. By rapidly removing the solvent, the diffusion of the active principle is prevented by the formation of a polymer barrier. Furthermore, the microspheres will be cured, thus preventing aggregation.

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The increase in the volume of the curing agent, which takes place gradually by additions at regular intervals, makes it possible to better extract the solvent for the microparticles.

The ratio of the volume of the curing agent to the volume of the solvent is between 5/1 and 180/1 and preferably between 15/1 and 120/1.

10

Combinations using acetic acid or N-methylpyrrolidone as solvent for the polymer require little curing agent to give solid microspheres, such that the ratio of the volume of the curing agent to the volume of the solvent is advantageously about 5/1 in this case.

20

The method for drying microparticles depends on the rigidity of the microspheres, the size and the volumes to be treated.

The tendency of the microparticles to aggregate after drying depends on their degree of hydration and on the residual amounts of solvent.

25 If drying in the open air is insufficient, placing under vacuum and/or increasing the temperature allow it to be completed, on condition that the microparticles can withstand the vacuum. However, it is necessary to

- 20 -

ensure that the drying rate and temperature are not too high, to prevent aggregation of the microparticles.

In the case of combinations using ethyl acetate as  
5 solvent for the polymer, the problem arises of choosing  
the method for separating out the microspheres.  
Specifically, the microspheres are still engorged with  
solvent after stirring for one hour in the curing  
agent. Filtration on a Millipore® system, through a  
10 filter of porosity 0.5  $\mu\text{m}$ , gives rise to a solid cake  
that is difficult to redisperse. Furthermore, the  
filter is then quickly clogged with the microspheres,  
which are still deformable. Separation by  
centrifugation on an aliquot of the solution did not  
15 give the hoped for result. In this case also, the  
microspheres aggregate and form a pellet that cannot be  
redispersed, even at a relatively low centrifugation  
speed. Another filtration technique using a fluted  
filter paper is found to be a solution. This process  
20 has the advantage of a large filtration surface and is  
performed at atmospheric pressure. However, the  
microspheres of smallest size are also prone to be  
adsorbed into the pores and gradually clog the filter.  
It is also difficult to recover all of the  
25 microspheres. As regards drying, this is carried out  
with a stream of compressed air and the microspheres  
are then left in ambient air.

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According to one embodiment of the invention, the solvent for the polymer is ethyl acetate, the nonsolvent is 2-propanol and the curing agent is a water/surfactant mixture, optionally a water/surfactant/alcohol mixture.

In this embodiment, the polymer is preferably a 75:25 PLAGA. The polymer concentration is between 1 and 5% (w/v) and preferably equal to about 4% (w/v). The coacervation is conducted at ambient temperature, preferably at a temperature below 4°C, more preferably less than or equal to -4°C, with mechanical stirring, preferably at 300 rpm.

The surfactant concentration is between 1 and 10% (v/v). The surfactant is Tween<sup>®</sup> 80. When an alcohol is combined with water, the surfactant concentration is set at between 1 and 10% approximately and the alcohol concentration is set at between 2.5 and 5% approximately. The alcohol is advantageously 2-propanol or 1,2-propanediol. The aqueous solution of curing agent is added in at least four portions. The curing operation lasts for at least 2 hours 30 minutes and for not more than 4 hours, and is carried out at ambient temperature, preferably at a temperature below 4°C, more preferably at 0.5°C, with mechanical stirring (500 rpm).

The nonsolvent/solvent ratio by volume is equal to 1/2.

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The curing agent/solvent ratio by volume is equal to 120/1.

5 The lower the curing temperature, the shorter the curing operation will be. Thus, when the temperature is below 4°C, a duration of 4 hours is sufficient. The duration is lowered to 2 hours 30 minutes when the temperature is 0.5°C.

10

According to another embodiment, the solvent is N-methylpyrrolidone, the nonsolvent is ethanol and the curing agent is a water/surfactant mixture. The polymer concentration is between 4 and 10% (w/v). The  
15 coacervation and curing operation are performed at ambient temperature, with magnetic stirring. The surfactant concentration is between 0.5 and 10% (v/v). The curing time is between 2 and 4 hours. The curing agent/solvent ratio by volume is equal to 40/1. The  
20 polymer is preferably Resomer RG<sup>®</sup> 502 or Resomer RG<sup>®</sup> 756.

The present invention is illustrated with the examples which follow, without limiting its scope.

**Example 1: Assessment of the polymer/solvent/coacervating agent/curing agent combinations.**

- 5 In this study, the poly( $\alpha$ -hydroxy acids) were assessed. Three copolymers of lactic acid and glycolic acid, in which the proportions of L- and D-lactides and glycolides are variable, were used. These are the following polymers, supplied by Boehringer Ingelheim:
- 10
- Resomer<sup>®</sup> RG 502 (Mw = 14,300 g/mol, Mn = 6900 g/mol), which comprises 25% L-lactide, 25% D-lactide and 50% glycolide,
  - 15 - Resomer<sup>®</sup> RG 756 (Mw = 89,800 g/mol, Mn = 35,200 g/mol), which contains 37.5% L-lactide, 37.5% D-lactide and 25% glycolide,
  - Resomer<sup>®</sup> RG 858 (Mw = 87,000 g/mol, Mn = 22,000 g/mol), which has 42.5% L-lactide, 42.5% D-lactide and 15% glycolide.
- 20

In view of the respective solvents for the polymers, the least toxic solvents were selected. They belong to  
25 solvents of category 2 or 3, defined by the classification of the ICH guidelines.

The miscibility of the solvent/nonsolvent pairs for the three polymers studied was then determined. Similarly,



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the nonsolvents for the polymers were chosen for their low toxicity.

Screening of the solvent/nonsolvent/curing agent combinations is performed in scintillation flasks, on small volumes of organic solutions of polymers at 1 or 4% (for N-methylpyrrolidone and 50:50 PLAGA exclusively) (m/v), 5 ml of organic solution of polymer are placed in a scintillation flask. The coacervating agent is then added until a cloudiness which persists on stirring, which is characteristic of the formation of coacervate, is obtained. The coacervate is observed at this step by optical microscopy. Next, 1 ml of this mixture is poured into 10 ml of an aqueous solution of surfactant. The presence or absence of microspheres is observed by optical microscopy.

**a) Coacervation tests with 50:50 PLAGA (Resomer® RG 502)**

20

The solvents in which 50:50 PLAGA is soluble are ethyl acetate, acetone, acetonitrile, acetic acid, dimethylacetamide, dimethylformamide, ethyl lactate, N-methylpyrrolidone and propylene carbonate. 50:50 PLAGA is insoluble in toluene, 2-propanol, glycerol, dioctyl adipate, 1,2-propanediol, xylene, diethyl carbonate and methyl ethyl ketone.

25

- 25 -

The combinations for which the formation of a coacervate and of microspheres is observed are the following:

- 5    -    ethyl acetate/2-propanol/water + Tween<sup>®</sup> 80,
- acetic acid/1,2-propanediol or 2-propanol/water +  
         Tween<sup>®</sup> 80,
- 10   -    N-methylpyrrolidone/ethanol/water + Tween<sup>®</sup> 80 or  
         2-propanol,
- N-methylpyrrolidone/methyl ethyl ketone or 2-  
         propanol/water + Tween<sup>®</sup> 80,
- 15   -    propylene carbonate/2-propanol/water optionally  
         with Tween<sup>®</sup> 80, ethanol or NaCl.

Acetic acid may be used as solvent for the polymer and  
20   to give rise to the formation of coacervate and of  
     correctly individualized microspheres, on condition  
     that water is used as curing agent. Effectively,  
     droplets of coacervate form in the acetic acid/2-pro-  
     panol combination, but in the presence of methyl ethyl  
25   ketone or 1,2-propanediol as curing agents, the  
     coacervate droplets do not conserve their spherical  
     shape and lumps of polymers are formed.

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The ethyl acetate/2-propanol/water + Tween<sup>®</sup> 80 combination gives good results, as does the N-methylpyrrolidone/ethanol/water + Tween<sup>®</sup> 80 combination. This study was completed by the investigation of curing  
5 agents. 1,2-Propanediol and 2-propanol are found to be good candidates. However, a reduced capacity for extracting the solvent may be noted with 1,2-propanediol than in the presence of 2-propanol.

10 Propylene carbonate is an advantageous solvent due to its low toxicity and also its partial solubility in water. The propylene carbonate/2-propanol combination makes it possible to dispense with water by virtue of the possibility of replacing the outer phase with a  
15 solvent or, at least, of limiting the diffusion of the active principle, by adding an electrolyte in the outer phase or by mixing water and a solvent.

**b) Coacervation tests with 75:25 PLAGA (Resomer<sup>®</sup>  
20 RG 756)**

The solvents in which 75:25 PLAGA is soluble are ethyl acetate, acetone, acetonitrile, acetic acid, dimethylacetamide, dimethylformamide, diethyl ether,  
25 methyl ethyl ketone and N-methylpyrrolidone.

75:25 PLAGA is insoluble in toluene, 2-propanol, glycerol, 1,2-propanediol, propylene carbonate, dioctyl adipate and triethyl citrate.

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The two solvents that give the best results are acetic acid and ethyl acetate. The studies were thus focused on assessing acetic acid/glycerol and acetic acid/1,2-propanediol combinations and also ethyl acetate/2-propanol and ethyl acetate/1,2-propanediol pairs.

The combinations for which the formation of individualized microspheres is observed are:

10

- ethyl acetate/1,2-propanediol/water + Tween<sup>®</sup> 80 or 2-propanol,

15

- acetic acid/glycerol or 1,2-propanediol/water + Tween<sup>®</sup> 80.

**c) Coacervation tests with 85:15 PLAGA (Resomer<sup>®</sup> RG 858)**

20 The solvents in which 85:15 PLAGA is soluble are ethyl acetate, acetone, acetonitrile, acetic acid, dimethylformamide, ethanolamine, ethylenediamine, methyl ethyl ketone, N-methylpyrrolidone, toluene and triethyl citrate. 85:15 PLAGA is insoluble in 2-propanol, glycerol, 1,2-propanediol, dioctyl adipate and xylene.

The combinations for which the formation of coacervate and microspheres is observed are:

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- ethyl acetate/2-propanol or 1,2-propanediol/water + Tween<sup>®</sup> 80,
- 5 - ethyl acetate/2-propanol/1,2-propanediol,
- ethyl acetate/1,2-propanediol/2-propanol,
- acetic acid/glycerol (water + Tween<sup>®</sup> 80) or 1,2-  
10 propanediol or 2-propanol,
- acetic acid or methyl ethyl ketone/1,2-  
propanediol/water + Tween<sup>®</sup> 80,
- 15 - acetic acid/2-propanol/1,2-propanediol,
- N-methylpyrrolidone or methyl ethyl ketone/2-  
propanol/water + Tween<sup>®</sup> 80,
- 20 - methyl ethyl ketone/1,2-propanediol/2-propanol.

**Example 2: Production of microspheres not containing active principle, by varying the coacervation parameters**

25

In a first stage, the operating conditions are established for the preparation of microspheres without active principle, so as to produce particles of the desired size. The effect of various factors, for

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instance the volume of coacervating agent added, the volume of curing agent, the type and speed of stirring, and the method for collecting the microspheres, is studied.

5

The polymer is dissolved in 50 ml of organic solvent (Beaker No. 1) to give a 1% solution (w/v). The polymer concentration is brought to 4% (w/v) when the solvent is N-methylpyrrolidone. With stirring, the coacervating agent is added until a stable and visible coacervate is obtained. The mixture is then poured into a solution of curing agent supplemented with a surfactant (Beaker No. 2), with stirring. The microspheres are then recovered by filtration. The combinations tested are those selected in example 1.

15

**a) With 50:50 PLAGA (Resomer<sup>®</sup> RG 502)**

The various protocols corresponding to each of the combinations selected are summarized in table 1.

20

TABLE 1

Procedures defined for the formulations of microspheres not containing active principle, based on 50:50 PLAGA								
Combinations Solvent/nonsolvent (with water + surfactant as curing agent	Volume of nonsolvent	Stirring Beaker 1	Speed Beaker 1	Stirring Beaker 2	Speed Beaker 2	Volume of curing agent	Filtration method	Size of the microspheres
Ethyl acetate/2-propanol	9 ml	Magnetic	500 rpm	Magnetic	700 rpm	750 ml	Fluted filter	40 μm
Acetic acid/1,2-propanediol	16 ml	Ultrasound		Magnetic	1100 rpm	250 ml	Fluted filter	24 μm
Acetic acid/2-propanol	16 ml	Ultrasound		Magnetic	1400 rpm	250 ml	Fluted filter	67 μm
N-Methylpyrrolidone/ethanol	31 ml	Mechanical	200 rpm	Magnetic	1000 rpm	1000 ml	Centrifugation	31 μm
N-Methylpyrrolidone/2-propanol	40 ml	Magnetic	300 rpm	Magnetic	1000 rpm	500 ml	Fluted filter	70 μm

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**b) Application to 75:25 PLAGA (Resomer<sup>®</sup> 756)**

Microspheres are obtained under the conditions described in table 2 with ethyl acetate as solvent.

5 Three stirring possibilities appear for the ethyl acetate/1,2-propanediol/water + Montanox<sup>®</sup> 80 or Tween<sup>®</sup> 80 combination: agitation by ultrasound, mechanical paddle stirring and magnetic stirring. There are no appreciable differences in the morphology or size of

10 the microspheres that can justify the choice of one of these methods.



TABLE 2

Procedures defined for the formulations of microspheres not containing active principle, based on 75:25 PLGA								
Combinations Solvent/nonsolvent (with water + surfactant as curing agent	Volume of nonsolvent	Stirring Beaker 1	Speed Beaker 1	Stirring Beaker 2	Speed Beaker 2	Volume of curing agent	Filtration method	Size of the microspheres
Ethyl acetate/1,2-propanediol	15 ml	Ultrasound		Magnetic	1100 rpm	750 ml	Fluted filter	20 μm
Ethyl acetate/1,2-propanediol	15 ml	Mechanical	400 rpm	Magnetic	1100 rpm	750 ml	Fluted filter	25 μm
Ethyl acetate/2-propanol	16 ml	Magnetic	900 rpm	Magnetic	1100 rpm	800 ml	Fluted filter	32 μm

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**Example 3: Production of microspheres containing 5-fluorouracil (5-FU) as active principle**

The tests are performed with the combinations of example 1 a) and b), which led to the observation of a coacervate.

**a) Effect of the active principle on the coacervation**

10 The active principle at low concentration (5% by weight) forms with the polymer solution a homogeneous dispersion. The stability of the dispersion depends on the solvents; thus, N-methylpyrrolidone, which is a viscous solvent, leads to a better stability of the  
15 dispersion and the losses of antimitotic agent by decantation or adsorption onto the walls of the beakers are reduced.

The use of 1,2-propanediol as coacervating agent  
20 further stabilizes the system by increasing the viscosity of the medium.

**b) Production yield**

25 This ranges from 15 to 100% depending on the combinations. The combinations using ethyl acetate as solvent for the polymer give the best yields, close to 100%.

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**c) Yield and degree of encapsulation**

When the theoretical contents of active principle are increased, the encapsulation yield is very low.

5

Changing to ultrasound makes it possible to individualize the active principle particles. When low concentrations are used, the dispersion is homogeneous and encapsulation yields of 70% are achieved.

10

**Example 4: Study of the N-methylpyrrolidone/ethanol/water + surfactant combination**

Among the N-methylpyrrolidone-soluble polymers tested, the following will be selected:

- Resomer<sup>®</sup> RG 502 (Boehringer Ingelheim), which is a 50:50 D,L-PLAGA,
- Resomer<sup>®</sup> RG 756 (Boehringer Ingelheim) and Phusiline (Saint-Ismier), which are 75:25 D,L-PLAGAs.

The initial formulation of the microparticles uses an organic solution of polymer at 4 or 10% (w/v) for a volume of solvent of 5 ml. The coacervation is performed with magnetic stirring. The nonsolvent for the polymer is added using a micropipette, milliliter by milliliter. As soon as cloudiness appears in the solution, a sample is taken and observed by optical

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microscopy. One minute after the appearance of the cloudiness, the coacervation medium is poured dropwise into 200 ml of curing agent (water + Tween<sup>®</sup> 80 or PVA). The curing of the microparticles is performed with  
5 mechanical stirring. A further observation of the microparticles by optical microscopy is carried out during the curing. The microparticles are then filtered under vacuum, or at atmospheric pressure on filter paper should it be impossible to filter under vacuum  
10 (virtually immediate clogging of the filter or excessively long filtration time). Finally, the microparticles are observed just after filtration and after lyophilization.

15 The microparticles obtained with the NMP/ethanol/water + surfactant combination are, by optical microscopy, spherical, smooth and uniform, whatever the stage of the formulation and whatever the type of polymer.

20 For all the batches prepared, the filtration after curing was performed on filter paper and at atmospheric pressure. The size of the microparticles is between 30 and 50  $\mu\text{m}$ . Numerous microparticles about 5  $\mu\text{m}$  in diameter are also observed.

25

◦ **Effect of the type of polymer and its concentration**

The microparticles obtained all have the same appearance by optical microscopy.

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The best yields are obtained with Resomer<sup>®</sup> RG 502 and Resomer<sup>®</sup> RG 756 under the following conditions (table 3).

TABLE 3

Type of polymer	Polymer concentration (w/v)	Type of curing agent	Concentration of the surfactant in the curing agent (v/v)	Curing time	Temperature during the coacervation	Temperature during the curing	Manufacturing yield
RG 502	10%	PVA	0.5%	3 h 30	Ambient	Ambient	23.8%
RG 502	10%	Tween® 80	1%	3 h 30	Ambient	< 4°C	35.9%
RG 756	4%	Tween® 80	1%	2 h	Ambient	Ambient	49.0%
RG 756	4%	Tween® 80	5%	2 h	Ambient	Ambient	38.6%

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◦ **Effect of the nature of the curing agent and of the curing time**

5 The type of curing agent (water, water + Tween<sup>®</sup> 80, or water + PVA) for the same polymer 50:50 PLAGA (RG 502) and at ambient temperature, has no influence either on the filtration or on the yield for the manufacture of the microparticles, which remains between 17.5 and 23.8%.

10

The curing time, for the same polymer 50:50 PLAGA (RG 502) makes it possible to increase the manufacturing yield by about 10%, at ambient temperature.

15 ◦ **Effect of the temperature**

a) During curing

20 Curing at a temperature below 4°C made it possible to double the manufacturing yield of the microparticles, for 50:50 PLAGA (RG 502): 35.9 as opposed to 17.5%.

b) During coacervation

25 The manipulations performed entirely at a temperature below 4°C have a maximum manufacturing yield of 10.5%.

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The N-methylpyrrolidone/ethanol/water + surfactant combination leads to the formation of microparticles of a satisfactory appearance by optical microscopy.

5 **Example 5: Microspheres containing progesterone or budesonide**

Progesterone and budesonide are hydrophobic active principles that are soluble in ethyl acetate.

10

The ethyl acetate/2-propanol/water + Tween<sup>®</sup> 80 combination is used, and 75:25 D,L-PLAGA (Phusis, Saint-Ismier) is used as polymer.

15 The manufacturing yields are all greater than 90%.

The results obtained are collated in table 4.



TABLE 4

Batch	Polymer Concen- tration (w/v) and mass AP	Coacervation			Curing					Manu- factur- ing yield	Particle size ( $\mu$ m)
		Method for adding the nonsol- vent	Tempe- rature	Stir- ring	Type	Conc. surfac- tant	Volume	Tempe- rature	Time	Stir- ring	
1	4% proges- terone 28.5 mg (solu- tion)	200/200 $\mu$ l 1 min between each addition	-4°C	Mechan- ical 300 rpm	Tween <sup>®</sup> 80	10%	300 ml +150 ml +150 ml (filtr.) +300 ml	0.5°C	1 h +30 min + 1 h (filt. ) +30 min	Mechan- ical 500 rpm	28.0 ± 12.5
2	4% budeso- nide 30.1 mg (solu- tion)	200/200 $\mu$ l 1 min between each addition	-4°C	Mechan- ical 300 rpm	Tween <sup>®</sup> 80	10%	300 ml +150 ml +150 ml (filtr.) +300 ml	0.5°C	1 h +30 min + 1 h (filt. ) +30 min	Mechan- ical 500 rpm	25.3 ± 9.2

Batch	Polymer Concen- tration (w/v) and mass AP	Coacervation			Curing					Manu- factur- ing yield	Particle size ( $\mu$ m)
		Method for adding the nonsol- vent	Tempe- rature	Stir- ring	Type	Conc. surf- fac- tant	Volume	Tempe- rature	Time	Stir- ring	
3	4% budeso- nide 30.8 mg (solu- tion)	200/200 $\mu$ l 1 min between each addition	-4°C	Mechan- ical 300 rpm	Tween <sup>®</sup> 80	10%	300 ml +150 ml +150 ml (filtr.) +300 ml	0.5°C	1 h +30 min + 1 h (filt.) ) +30 min	Mechan- ical 500 rpm	>99.9%  20.8 ± 8.7
4	4% budeso- nide 29.8 mg (solu- tion)	200/200 $\mu$ l 1 min between each addition	-4°C	Mechan- ical 300 rpm	Tween <sup>®</sup> 80	10%	300 ml +150 ml +150 ml (filtr.) +300 ml	0.5°C	1 h +30 min + 1 h (filt.) +30 min	Mechan- ical 500 rpm	97.6%  22.5 ± 9.4

\* Filtration under vacuum, Nylon filter

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The yields and degrees of encapsulation of the active principles are given in the table below and are calculated as follows:

5 - Ttheo. = [weight of active principle/(weight of polymer + weight of active principle)] × 100,

- Texp. = [weight of active principle/weight of dry microparticles] × 100.

10

They make it possible to calculate the encapsulation yield Y:

- Y = [Texp./Ttheo.] × 100

15

Yields and degrees of encapsulation of progesterone and budesonide with 75:25 PLAGA (Phusis)				
Batch	Theoretical degree	Experimental degree	Encapsulation yield	Degree of residual moisture
1 (progesterone)	12.4%	8.2%	66.1%	11.9%
2 (progesterone)	13.1%	9.5%	72.5%	-
3 (budesonide)	13.3%	8.0%	60.1%	-
4 (budesonide)	13.0%	8.8%	67.7%	16.4%

The ethyl acetate/2-propanol/water + surfactant combination leads to the formation of microparticles with a satisfactory appearance by optical microscopy.

20 On the other hand, the microparticles obtained with

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this solvent/nonsolvent pair are more fragile and are dried in the open air, thus at ambient temperature and at atmospheric pressure, so as not to be irreversibly aggregated. The manufacturing yields are close to 100%.

- 5 The aggregation is the main problem encountered with this solvent/nonsolvent/curing agent combination. It may be reduced by modifying the various parameters of the formulation. The temperature at which the coacervation is carried out is specially an essential
- 10 factor: when lowered to  $-4^{\circ}\text{C}$ , it makes it possible to set the polymer and to rigidify the microparticles, which thus become individualized and no longer stick together.